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Inhalation Pharmacokinetics of Isoprene in Rats and Mice

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Studies on inhalation pharmacokinetics of isoprene were conducted in rats (Wistar) and mice ($B6C3F_1$) to investigate possible species differences in metabolism of this compound. Pharmacokinetic analysis of isoprene inhaled by rats and mice revealed saturation kinetics of isoprene metabolism in both species. For rats and mice, linear pharmacokinetics apply at exposure concentrations below 300 ppm isoprene. Saturation of isoprene metabolism is practically complete at atmospheric concentrations of about 1000 ppm in rats and about 2000 ppm in mice. In the lower concentration range where first-order metabolism applies, metabolic clearance (related to the concentration in the atmosphere) of inhaled isoprene per kilogram body weight was 6200 mL/hr for rats and 12,000 mL/hr for mice. The estimated maximal metabolic elimination rates were 130 μmole/hr/kg for rats and 400 μmole/hr/kg for mice. This shows that the rate of isoprene metabolism in mice is about two or three times that in rats.

When the untreated animals are kept in a closed all-glass exposure system, the exhalation of isoprene into the system can be measured. This shows that the isoprene endogenously produced by the animals is systemically available within the animal organism. From such experiments the endogenous production rate of isoprene was calculated to be 1.9 μmole/hr/kg for rats and 0.4 μmole/hr/kg for mice. Our data indicate that the endogenous production of isoprene should be accounted for when discussing a possible carcinogenic or mutagenic risk of this compound.

Introduction

Isoprene (2-methyl-1,3-butadiene) is used extensively in the manufacture of synthetic elastomers and predominantly in the production of *cis*-1,4-polyisoprene (*2*). Isoprene is structurally related to 1,3-butadiene, a major component in synthetic rubber, which has been shown to be carcinogenic in mice (*3*) and rats (*4*) and genotoxic in mice (*5*).

In liver microsomal incubations of mouse, rat, rabbit, and hamster (*6,7*) isoprene is metabolized by cytochrome P-450 enzymes to the corresponding monoepoxides 3,4-epoxy-3-methyl-1-butene (half-life 75 min) and 3,4-epoxy-2-methyl-1-butene (half-life 73 hr) at a ratio of about 4:1. When added to mouse liver microsomal incubations (*6*) the minor epoxide metabolite 3,4-epoxy-2-methyl-1-butene was further oxidized to isoprene dioxide (2-methyl-1,2,3,4-diepoxybutane).

In contrast to butadiene (*8*), isoprene or its monoepoxides were not mutagenic in *S. typhimurium*, either in the presence or absence of rat liver S9 (*9*). Isoprene diepoxide (2-methyl-1,2,3,4-diepoxybutane), however, appears to be as potent a mutagen in *S. typhimurium* as diepoxybutane (*9*). Furthermore, cytogenetic damage could be observed in $B6C3F_1$ mice after exposure of the animals to isoprene (*10*). In addition, 2-methyl-1,2,3,4-diepoxybutane could be characterized by vacuum line cryogenic distillation in the blood and other tissues of rats after exposure of the animals to ^{14}C-isoprene (*11*).

The purpose of our studies was to provide comparative pharmacokinetic data on the metabolism of inhaled isoprene in rats and mice, as previously obtained for 1,3-butadiene (*12*). Therefore, comparative studies in rats (Wistar) and mice ($B6C3F_1$) on inhalation pharmacokinetics of isoprene have been conducted. Furthermore, exhalation by untreated animals of endogenously formed isoprene was measured, and the rate of endogenous production of this compound was calculated for both species.

Methods

The methodological details of this investigation have already been published (*13*). Thus, only a brief summary is given here of the methods used. [See also Laib et al. (*12*).]

Male Wistar rats (200–250 g) and male $B6C3F_1$ mice

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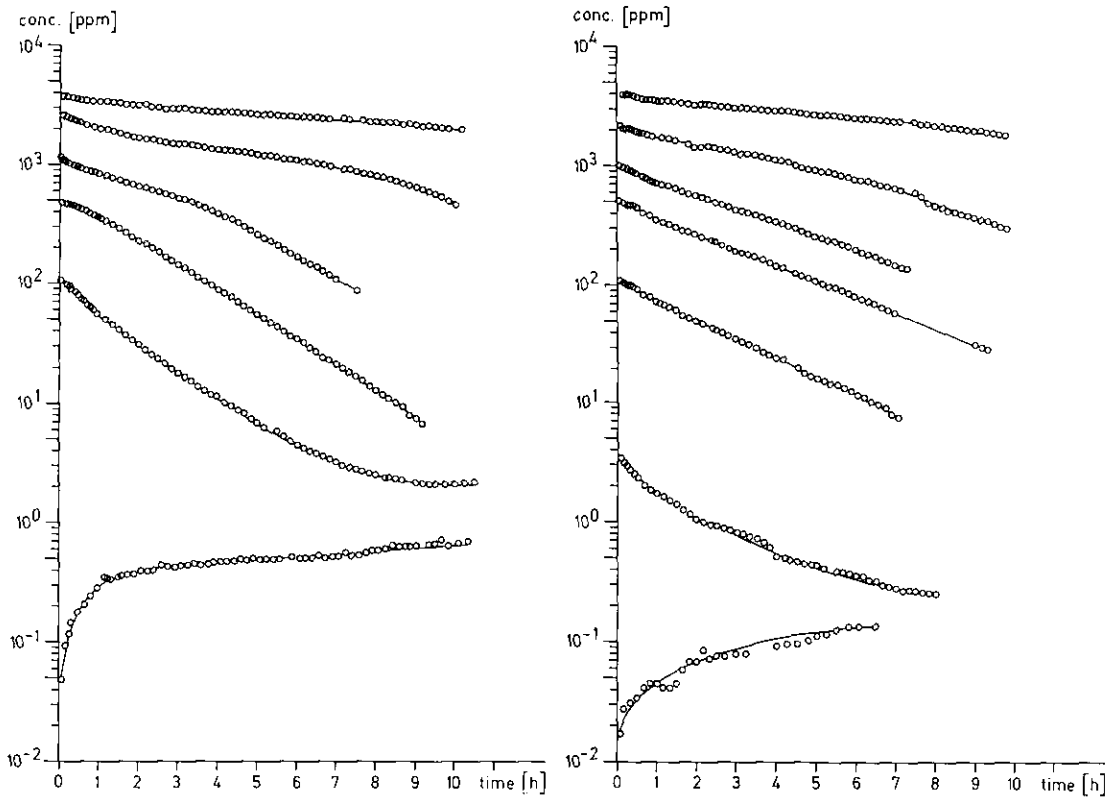

FIGURE 1. The top line of each graph refers to the time course of isoprene concentrations in the gas phase of a closed 6.4 L desiccator jar chamber occupied by two Wistar rats (left) or five B6C3F$_1$ mice (right). Individual experiments with different initial isoprene concentrations between 5 and 5000 ppm. The bottom line of each graph refers to the time course of isoprene concentrations exhaled by untreated animals into the gas phase of the closed exposure system (6.4 L) occupied by two Wistar rats (left) or five B6C3F$_1$ mice (right).

(25–30 g) were used in these experiments. Usually two rats or five mice were placed in a closed 6.4 L desiccator jar chamber, equipped with 135 g soda lime for CO_2 absorption and an oxygen supply (*12*). The animals were exposed to initial concentrations between about 5 ppm and 5000 ppm isoprene. Initial concentrations were adjusted by either injecting a gaseous mixture of isoprene with air (5–1000 ppm) or by direct injection of the volatile liquid (1000 ppm) into the closed exposure system. Concentration changes of the compound in the gas phase of the system were measured by gas chromatography (Fig. 1, top). Similarly, exhalation and accumulation of isoprene endogenously produced by untreated animals, while in the closed exposure system was determined (Fig. 1, bottom).

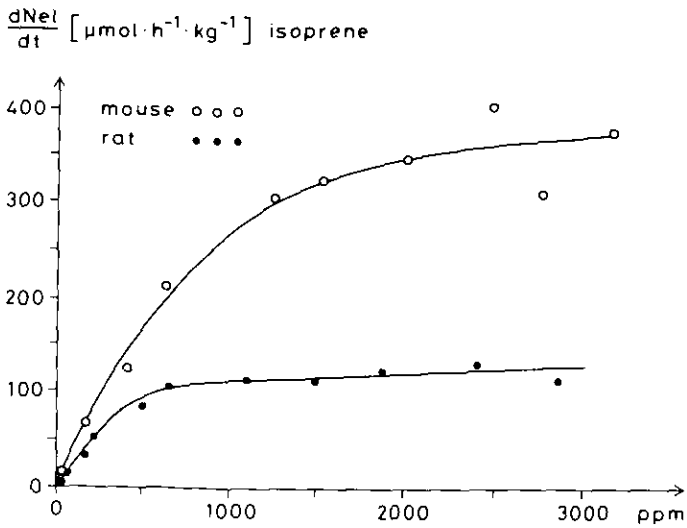

FIGURE 2. Metabolic elimination rates of B6C3F$_1$ mice (○) and Wistar rats (●) depending on atmospheric concentrations of isoprene.

Kinetic parameters were determined from the concentration time-courses thus obtained, based on a two-compartment, pharmacokinetic model developed by Filser and Bolt (*14,15*). The gas phase in the desiccator with volume V_1 represented compartment 1 (Cp1), the animals with volume V_2 represented compartment 2 (Cp2) [see model in Laib et al. (12)]. The full details of the analytical procedures and the pharmacokinetic analysis have been presented elsewhere (*13–15*).

Results and Discussion

Inhalation Pharmacokinetics of Isoprene

Starting from different initial concentrations between 5 and 5000 ppm, the time-dependent decline of isoprene

in the exposure system, occupied by rats or mice, was investigated (*13*). The five decline curves observed in these experiments for rats or mice (Fig. 1, top) become flatter at higher exposure concentrations, indicating saturable metabolism of isoprene in both species. Below concentrations of about 300 ppm isoprene, metabolism is proportional to the atmospheric concentration of the substance, and the elimination of isoprene by rats or mice can be described by a first-order process. At higher atmospheric concentrations saturation kinetics become apparent in both species. Saturation of isoprene metabolism is practically complete at atmospheric concentrations of about 1500 ppm in rats and at about 2000 ppm in mice. At higher concentrations the increase is negligible in the metabolic rate of isoprene observed for both species.

The pharmacokinetic parameters for distribution and metabolism of isoprene were determined from the concentration-decline curves obtained (*14,15*) (Table 1). They show, in principle, that the rate of isoprene metabolism in mice is about two to three times that in rats. In the lower concentration range, where first-order metabolism applies, metabolic clearance per kg body weight was 12,000 mL/hr for mice and 6,200 mL/hr for rats. The estimated maximal metabolic elimination rates were 400 μmole/hr/kg for mice and 130 μmole/hr/kg for rats.

Accumulation of isoprene in the organism is determined by the rates of uptake via inhalation, exhalation, and metabolism. Isoprene accumulates in the organism as long as the rates of inhalation exceed the rates of exhalation and metabolism. At high concentrations, when metabolizing enzymes are saturated, accumulation is determined only by the rates of inhalation and exhalation, whereas metabolism becomes negligible. At such conditions accumulation is determined by the thermodynamic partition coefficient that represents the concentration equilibrium between the animal organism and the atmosphere (*14*). Accumulation of isoprene is very similar in both species investigated (7.8 times in rats and 7.0 times in mice) and is related to the solubility of isoprene in the tissues of the animals. A similar thermodynamic partition coefficient for isoprene of about 7.9 can be estimated by means of the Ostwald partition coefficients of isoprene for olive oil/air, and saline/air, respectively (*16*).

In the lower concentration range where first-order metabolism applies (< 300 ppm), the concentration ratios of isoprene between the animal organism and atmosphere are much lower than could be expected from the partition coefficients. In this concentration range only limited accumulation is observed, and metabolic clearance in both species is similar to the clearance of uptake of isoprene from the gas phase. This indicates that the overwhelming part of isoprene entering the animal organism is metabolized. A comparison of the clearances of uptake (V_1k_{12}) and the clearances of metabolism ($V_2K_{st}k_{el}$) reveals, that only a minor part (about 15% in rats and about 25% in mice) of isoprene taken up by the animals is exhaled unchanged.

Figure 2 shows the metabolic elimination curves of isoprene for rats and mice, calculated for conditions of exposure in an open ($V_1 \longrightarrow \infty$) exposure system (*14*). Up to ambient concentrations of about 300 ppm isoprene, the metabolic elimination of isoprene is proportional to the exposure concentration in mice and rats. Above about 1000 ppm in rats and 2000 ppm in mice, saturation of isoprene metabolism is practically complete. A comparison of the metabolic elimination rates of both species at different exposure concentrations reveals that the metabolic elimination rate of isoprene in

Table 1. Pharmacokinetic parameters for distribution and metabolism of isoprene (2-methyl-1,3-butadiene) in mice ($B6C3F_1$) and rats (Wistar) related to 1 kg body weight. [For definition of parameters see text and references (*13,14*)].[a]

Parameter	Rats[b]	Mice[b]	Dimension
Thermodynamic partition coefficient (whole body/air); K_{eq}[c]	7.8 ± 3	7.0 ± 2	$\frac{\text{nL gas/mL tissue}}{\text{ppm in atmosphere}}$
Concentration ratio in steady state[c] (whole body/air); K_{st}[c]	1.2 ± 0.4	1.7 ± 0.6	$\frac{\text{nL gas/mL tissue}}{\text{ppm in atmosphere}}$
Clearance of uptake from the atmosphere (related to the concentration in the atmosphere); V_1k_{12}[c]	7,300 ± 2,000	16,000 ± 3,000	mL/hr
Clearance of metabolism[d] (related to the concentration in the atmosphere); $V_2K_{st}k_{el}$[c]	6,200 ± 1,000	12,000 ± 3,000	mL/hr
Clearance of exhalation (related to the concentration in the body); V_2k_{21}[c]	940 ± 300	2,300 ± 1,000	mL/hr
Half-life[d]; $ln2/(k_{el} + k_{21})$[c]	6.8 ± 2.4	4.4 ± 1.5	*min*
Maximal rate of metabolism; (V_{max})[e]	130 (Fig. 2)	400 (Fig. 2)	μmole/hr/kg
Endogenous production rate[e]; dNpr/dt[e]	1.9 ± 0.8	0.4 ± 0.2	μmole/hr/kg
Rate of metabolism of endogenously produced isoprene[e]	1.6 ± 0.7	0.3 ± 0.2	μmole/hr/kg

[a]Calculations for: 1 kg body weight (V_2 = 1000 mL); dynamic (open) exposure system [$V_1 \longrightarrow \infty$; according to Filser and Bolt (*14,15*)].
[b]Mean value ± SD of three exposures with two rats, five mice, each.
[c]Calculated for the systemically available isoprene.
[d]Pharmacokinetic constants calculated according to the two-compartment model (*14,15*).
[e]Between 50 and 250 ppm (rat) and between 10 and 300 ppm (mouse).

Table 2. Species differences in exhalation of isoprene endogenously produced.

Species	Rate of exhalation, μmole/hr/kg	Amount exhaled/24 hr (per individual)
Rat, 200 g	0.3	0.1 mg
Mouse, 30 g	0.1	0.005 mg
Man, 75 kg[a]	0.015–0.03	2–4 mg

[a]Data calculated from Gelmont et al. (*1*).

mice is about two to three times that in rats, depending on the exposure concentrations applied.

Exhalation of Isoprene by Untreated Animals

When untreated rats or mice are kept in the closed exposure system, exhalation of isoprene into the air of the system can be measured. Figure 1 (bottom curve) shows, that after an initial increase, isoprene exhaled reaches a plateau concentration of about 0.8 ppm for rats and about 0.2 ppm for mice. This shows that isoprene is endogenously produced by the animals and it is systemically available within the animal organism. From this experiment, the rate of endogenous production of isoprene can be calculated as 1.9 μmole/hr/kg for rats and 0.4 μmole/hr/kg for mice. Under these particular conditions the metabolic rate for endogenously produced isoprene is estimated to be about 1.6 μmole/hr/kg in rats and 0.3 μmole/hr/kg in mice.

The rate of production of endogenous isoprene in rats and mice exceeds that of *n*-pentane or ethane by two to three orders of magnitude (*17*). Isoprene is also the main hydrocarbon in human breath (*1,18*) and is exhaled by human subjects in amounts of 2 to 4 mg per day. A species comparison of the rates of exhalation of endogenously produced isoprene reveals that the exhalation rate of isoprene by man (related to kilogram body weight) is about one order of magnitude lower when compared to mouse or rat (Table 2).

Recent data on cytogenetic activity of isoprene in the bone marrow cells of $B6C3F_1$ mice [significantly elevated frequencies of sister chromatid exchange and micronuclear polychromatic erythrocytes after exposure of the animals to isoprene (*19*)] and similar toxicologic effects as previously observed for 1,3-butadiene in an ongoing inhalation exposure study with $B6C3F_1$ mice suggest that isoprene may have a carcinogenic potential in this species (*20*). Furthermore, isoprene-diepoxide was characterized in blood and other tissues of rats after exposure of the animals to ^{14}C-isoprene (*11*). These data stress the need for interstrain comparative studies on the metabolism and disposition of isoprene in rodents and man. Finally, our results indicate that the endogenous production of isoprene should be taken into account when discussing a possible carcinogenic or mutagenic risk of this compound.

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